



(19)

Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11)

EP 0 884 327 B1

(12)

# EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:  
14.03.2001 Bulletin 2001/11

(51) Int Cl.7: **C07K 7/02, C07K 14/00,  
A61K 38/04, A61K 38/16**

(21) Application number: 97304060.3

(22) Date of filing: 11.06.1997

(54) Dendritic lysine-based polypeptides for targeted drug delivery

Dendritische, lysinhaltige Polypeptide zur gezielten Arzneimittelabreicherung

## Polypeptides dendritiques à base de lysine pour apport ciblé de médicaments

(84) Designated Contracting States:  
CH DE ES FR GB IE IT LI NL

- **Wilderspin, Andrew F. The School of Pharmacy**  
London, WC1N 1AX (GB)
- **Toth, Istvan The School of Pharmacy**  
London, WC1N 1AX (GB)

(43) Date of publication of application:  
16.12.1998 Bulletin 1998/51

(74) Representative: Jones, Helen Marjorie Meredith  
Gill Jennings & Every,  
Broadgate House,  
7 Eldon Street  
London EC2M 7LH (GB)

(73) Proprietor: THE SCHOOL OF PHARMACY  
UNIVERSITY OF LONDON  
London WC1N 1AX (GB)

(56) References cited:  
WO-A-94/02506  
WO-A-95/00540

(72) Inventors:

- Florence, Alexander T.  
c/o The School of Pharmacy,  
London, WC1N 1AX (GB)
- Sakthivel, Thiagarajan The School of Pharmacy  
London, WC1N 1AX (GB)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

## Description

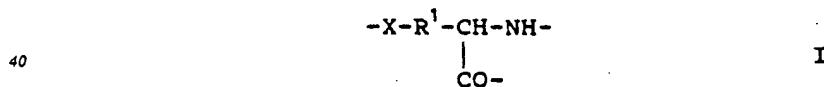
[0001] The present invention relates to polypeptide compounds which have dendritically linked units formed from amino acids having reactive groups, for instance carboxylic acid or amine groups, in their side chains. Each molecule 5 comprises two dendrons. To at least two of the terminal branches of one of the dendrons there are attached anchor groups, each of which comprises at least one lipophilic group. The terminal units of the at least one other dendron may be unconjugated or may be conjugated to ligands of various types. The dendrons are attached at a core which may include a linear oligopeptide, optionally having pendant sugar molecules.

[0002] Tam *et al*, in Proc.Nat.Acad.Sci.USA(1988) 85, 5409-5413 describe a compound including a dendritically 10 linked polylysine component, to the focal lysine of which is attached a lipophilic moiety, through a peptide bond to the carboxylic acid group of that lysine unit. To the terminal branches of the dendritic moiety there may be attached peptide antigens to provide an active ingredient for a vaccine having improved antigenicity. WO-A-95/00540 describes dendritic carriers, in which the dendrons include lysine or other di-amino carboxylic acids. The carrier may include a hydrophobic 15 group connected by a linker to the focal group of the dendritic molecule. Where the dendritic carrier is synthesised using solid state peptide synthetic methods, the hydrophobic group is linked following cleavage of the dendrimer from the carrier. Suitable hydrophobic groups are derived from fatty acids or fatty alcohols.

[0003] In WO-A-94/02506, Toth *et al* describe an improvement of Tam's invention, in which the anchor component 20 is formed from lipophilic amino acids. This allows the compound to be synthesised using conventional solid state peptide synthetic techniques, in the first stages of which the lipophilic amino acids are linked to form, for instance, a three unit linear oligopeptide, a focal lysine unit is joined to the final lipophilic amino acid and the dendritic core moiety is then linked to the two amine groups of the focal lysine unit. The peptide antigens may subsequently be synthesised directly onto the terminal branches of the dendritic core, all the steps being carried out without cleavage of the polypeptide from the solid substrate carrier. The synthetic process used to make Toth *et al*'s product required the use of starting 25 amino acid reagents with the same protecting group blocking the two amine groups of lysine reagents. Consequently during the steps in which the dendritic component is synthesised, the same reagent is added to each of the amine moieties.

[0004] In the product of Toth *et al* it was essential for the lipidic amino acids to be joined directly to one another by peptide bonds, and that a lipidic amino acid can be joined to a carrier substrate so that synthesis involves linkage of 30 that unit to the carrier by a peptide bond and linkage of another lipidic amino acid unit to the dendritic moiety by a peptide bond. Consequently solid state peptide synthesis methods can be used to conjugate each of the components of the final product to one another. By contrast, in Tam *et al*, whilst the dendritic polylysine and the peptide antigen can be synthesised using solid state peptide synthetic methods, the polylysine-polyantigen compound must be cleaved from the carrier substrate prior to conjugation to the lipophilic anchor moiety, through the carboxylic acid unit of the focal lysine group. The reagent, from which Tam's lipophilic anchor is synthesised, has only one reactive group.

[0005] A new dendritic compound according to the present invention comprises a core including a focal group from 35 which at least two dendrons extend, each dendron comprising dendritically linked amino acid units I



in which R<sup>1</sup> is C<sub>1-6</sub>-alkylene and X is selected from the group consisting of -O-, -S-, -NH- and -CO-, and each unit of 40 a dendron may have the same groups R<sup>1</sup> and X, and in which a first dendron had n (where n is 2) levels of dendritically linked amino acid units and 2<sup>n</sup> terminal branches, to p (where p is at least 2) of which terminal branches there are linked anchor groups



where Y is selected from -CO-, -NH-, -O- and -S-, provided that at least one of X and Y is -CO-,

45 R<sup>2</sup> is an organic group containing at least one C<sub>6-24</sub>-alkyl, -alkenyl, or -alkynyl group,  
 R<sup>3</sup> is selected from the group consisting of hydrogen, amine, blocked amine, hydroxyl, C<sub>1-24</sub> alkoxy, thiol, COOH, or an organic group containing at least one C<sub>6-24</sub>-alkyl, alkenyl or -alkynyl group, C<sub>1-6</sub>-alkanoyloxy, or C<sub>1-6</sub>-alkanamido,

and in which a second dendron has  $m$  (where  $m$  is in the range 3-5) levels of dendritically linked amino acid units of the formula I above in which the groups  $R^1$  and  $X$  may be the same as or different to one another and the same as or different to those of the amino acid units in the first dendron and  $2^m$  terminal branches, each of which is either unconjugated and is a group selected from  $NH_2$ ,  $N^+H_2R^{11}$ , in which  $R^{11}$  is hydrogen or  $C_{1-4}$  alkyl,  $COOH$ ,  $COO^-$ ,  $OH$  or  $SH$ , or is conjugated via the terminal  $-X-$ ,  $-NH-$  or  $-CO-$  group to a group  $R^{12}$  where  $R^{12}$  is a methyol group, an active ligand or an organic group comprising a sugar moiety.

[0006] In the present invention the focal group of the core is linked through covalent bond to the at least 2 dendrons. The core may include components other than the focal unit, for instance joined to the focal unit by one or more additional covalent bond. Preferably the focal group is an amino acid unit, for instance having the formula I

10



15

in which  $X$  and  $R^1$  are as defined above.

Preferably  $X$  is either  $-NH-$  or  $-CO-$ . Where it is  $-CO-$ , the two dendrons are attached to the two  $-CO-$  moieties. Where  $X$  is  $NH$ , the two dendrons are linked one each to the groups  $NH$ . Preferably the focal group is formed from lysine or ornithine.

20

[0007] The core may comprise units other than the focal group. Such units are preferably peptide linked amino acid based units. Additional core units may function merely as spacers, or may include functional groups such as lipophilic groups, hydrophilic groups or active ligands, for instance targeting groups. The compound may be attached to a resin through the focal unit, for instance via a spacer.

25

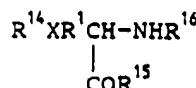
[0008] The present invention is made possible by the use in the synthesis of the dendritic compound of a reagent for forming the focal group which has at least three reactive groups, each of which can be sequentially reacted. Where, in the preferred embodiment of the invention, the focal group is an amino acid unit of the formula I where the group  $X$  is  $-NH-$ , the reagent from which the focal unit is derived has the two amine groups protected by two different protecting groups which are removable under different conditions. Each amine group can consequently be protected, activated and reacted in sequential series of reaction steps. This allows two different dendrons to be synthesised.

30

[0009] The present invention includes also a method for synthesising the novel compound in which a focal reagent which has two reactive groups is reacted in a first series of first dendron producing steps as follows:

1. an amino acid reagent of the formula II

35



40

in which  $R^1$  and  $X$  are as defined above,

45

$R^{14}$  is  $H$  when  $X$  is  $-O-$ ,  $-S-$  or  $-NH-$ ,  
 $OH$  when  $X$  is  $-CO-$ , or

is a protecting group,

$R^{15}$  is a carboxylic acid protecting group, hydroxyl, or a carboxylic acid activating group,

$R^{16}$  is  $H$ , an amine protecting group or an amine activating group,

provided that at least two of  $R^{14}$ ,  $R^{15}$  and  $R^{16}$  is other than an activating group and at least one of  $R^{14}$ ,  $R^{15}$  and  $R^{16}$  is other than a protecting group, is reacted

50

with the focal reagent, optionally after a step in which the desired reactive group of the focal reagent and/or one of the groups  $-XR^{14}$ ,  $-COR^{15}$  and  $-NHR^{16}$  is deprotected and/or activated whereby the reactive groups on the focal reagent reacts with one of the groups  $R^{14}X$ ,  $R^{15}CO$ - and  $R^{16}NH-$ ;

55

2. a second step in which both unreacted groups  $R^{14}X$ ,  $R^{15}CO$ - and  $R^{16}NH-$  of the product of the preceding step are, if necessary, deprotected and/or activated and reacted with at least two equivalents of a trifunctional reagent having the general formula II,



5

in which the groups R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> are as defined in step 1 and are the same or different as in the trifunctional reagent used in step 1;

3. One repeat of step 2, using at least four equivalents of trifunctional reagent; and
4. an anchor group attachment step in which at least two of the four groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH are, if necessary, deprotected and/or activated, and reacted with a lipophilic reagent of the formula III

10

15



in which Y, R<sup>2</sup> and R<sup>3</sup> are as defined above, and R<sup>17</sup> is OH or a carboxylic acid activating group, where Y is -CO- or is H or an amine, hydroxyl or thiol activating group, respectively, where Y is -NH-, -O- or -S-, whereby the said at least two groups react with R<sup>17</sup>Y-to conjugate

20

25

groups to the X, CO- or NH-; and

a second dendron forming series of reaction steps in which

- 30 1. the other of the reactive groups of the focal reagent is, in a step separate to step 1 mentioned above, reacted with an amino acid reagent of the formula II

35



in which R<sup>1</sup> and X are as defined above,

40

R<sup>14</sup> is H when X is -O-, -S- or -NH-,  
OH when X is -CO-, or  
is a protecting group,

R<sup>15</sup> is a carboxylic acid protecting group, hydroxyl, or a carboxylic acid activating group,

R<sup>16</sup> is H, an amine protecting group or an amine activating group,

45

provided that at least two of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than an activating group and at least one of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than a protecting group,

with the focal reagent, optionally after a step in which the desired reactive group of the focal reagent is deprotected and/or activated whereby the other of the reactive groups on the focal reagent reacts with one of the groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH-;

50

2. a second step in which both unreacted groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH- of the product of the preceding step are, if necessary, deprotected and/or activated and reacted with at least two equivalents of a trifunctional reagent having the general formula II,

55

5

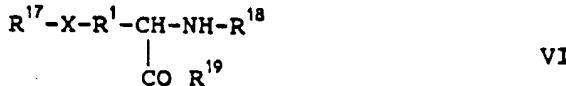


in which the groups R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> are as defined in step 1 and are the same or different as in the trifunctional reagent used in step 1;

3. (m-1) repeats of step 2, using in each case at least 2<sup>(r+1)</sup> equivalents of trifunctional reagent for the r<sup>th</sup> repeat of step 2, until m levels of dendritically linked amino acids have been formed, where m is in the range 3 to 5.

[0010] In a preferred reaction there is a preliminary step of reacting a focal reagent of the formula VI

15



20 in which R<sup>17</sup>, R<sup>18</sup> and R<sup>19</sup> are selected from the same groups as R<sup>14</sup>, R<sup>16</sup> and R<sup>15</sup>, respectively, as defined above, with a substrate having a pendant group which is capable of reacting with one of the groups X R<sup>17</sup>, -NH R<sup>18</sup> and -CO R<sup>19</sup>, optionally after deprotection and/or activation of the said pendant group or said one of the groups of the focal reagent, whereby the focal reagent is bound to the substrate.

[0011] In the process, the series of reactions used to form the dendron having lipophilic moieties R<sup>2</sup> may be carried out before or after the series of reactions to form the other dendron. Thus the reference to the first series of steps and

25 second series of steps does not, unless the context makes it explicit, imply an order of carrying out the said series.

[0012] In the invention, in the lipophilic component -Y-CH(R<sup>2</sup>)R<sup>3</sup>, R<sup>2</sup> is preferably selected from C<sub>6-24</sub>-alkyl, -alkenyl or -alkynyl, or is a group IV

30



in which R<sup>4</sup> is a bond or a C<sub>1-6</sub>-alkylene group,

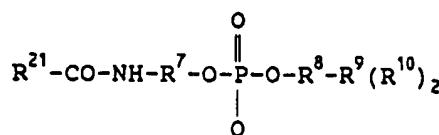
35

R<sup>5</sup> is hydrogen, a C<sub>1-6</sub>-alkyl or a C<sub>6-24</sub>-alkanoyl group or a group CH<sub>2</sub>SCH<sub>2</sub>CH(OCOR<sup>20</sup>)CH<sub>2</sub>OCOR<sup>20</sup>, in which R<sup>20</sup> is a C<sub>6-24</sub>-alkyl, -alkenyl or -alkynyl group,

R<sup>6</sup> is hydrogen or a C<sub>6-24</sub>-alkyl, -alkoxy, -alkanoyl or -alkanoyloxy group or R<sup>2</sup> is a group

40

45



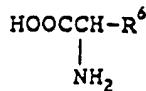
in which R<sup>8</sup>, R<sup>21</sup> and R<sup>7</sup> are each C<sub>2-6</sub>-alkylene

R<sup>9</sup> is glycerol and

50 each group R<sup>10</sup> is independently selected from C<sub>6-24</sub>-alkyl, -alkenyl, -alkynyl, -alkanoyl, -alkenoyl or -alkynoyl, provided that R<sup>5</sup> and R<sup>6</sup> cannot both be groups selected from hydrogen, lower alkyl, alkenyl and alkynyl groups.

[0013] Where the group R<sup>2</sup> is a group of the formula IV, and especially where R<sup>4</sup> is a bond, the compound is derived from a lipidic amino acid of the formula V

55



5

wherein  $\text{R}^6$  is as defined above.

[0014] In the step in which a lipidic amino acid is conjugated to the terminal branches of the first dendron whichever of the COOH and NH<sub>2</sub> group is not desired to react with the terminal branch is generally blocked by an appropriate protecting group.

[0015] It may often be more convenient to use, instead of the lipidic amino acid of the formula V, a monofunctional reagent to provide the anchor moieties, for instance a fatty acid or fatty amine.

[0016] The dendritic compound of the invention may be bound to a solid support, for instance a resin used as the solid peptide synthesis support. Thus the core is joined to the solid support, for instance a resin, through the focal unit, optionally via a spacer, for instance an oligopeptide spacer. The compound may be cleaved from the support prior to use, optionally after having reacted further any of the underivatised terminal branches. Thus the unreacted terminal branches may be in the form of free or protected carboxylic acid, amine, hydroxyl or thiol groups.

[0017] In a preferred aspect of the invention the dendritic compound has several terminal primary amino groups or is in the form of the corresponding ammonium salt. Usually all the terminal groups of the second dendron are amine or ammonium groups.

[0018] In a further preferred embodiment of the product of the invention, at least some of the terminal groups of the second dendron are attached to an organic group comprising a sugar molecule.

[0019] Generally it is preferred for all of the terminal branches of the first dendron to be provided with lipophilic anchor moieties. It is found that two or four such moieties are adequate to provide appropriate levels of lipophilicity to the compound as a whole.

[0020] The second dendron has at least three levels of dendritically linked amino acid units, preferably four or, sometimes five levels of dendritically linked amino acid units (that is, m is 3 to 5). Where there are five or more levels of dendritically linked amino acid units, steric hindrance may prevent full dendritic linkage of groups, for instance further dendritic moieties, to the terminal units. Consequently it is preferred for there to be no more than five, and preferably four, levels of dendritically linked amino acid units.

[0021] As indicated below in the detailed examples, it has been found that the dendrimer of the present invention having four anchor groups being lipidic amino acid units joined to the amine terminal groups of the first dendron, and with free amino groups at each of the terminal groups of 3-, 4- and 5- level dendritically linked amino acid units for the second dendron have reduced toxicity as determined by erythrocyte lysis, as compared to a lipid peptide dendrimer as described in our earlier application WO-A-94/02506 comprising a linear oligopeptide anchor moiety of three lipidic amino acids joined to the focal lysine of a dendrimer having the equivalent number of levels of dendritically linked amino acid (lysine) units.

[0022] The compound of the invention has a similar utility to those described in WO-A-94/02506. Thus, to the terminal branches of the second dendron, there may be conjugated peptide antigens, drug moieties, targeting moieties, for instance antibodies or sugar groups, or other groups providing increased levels of hydrophilicity (for instance sugar molecules, polyethylene glycol molecules or ionic moieties).

[0023] The invention is illustrated further in the following examples.

#### MATERIALS AND METHODS

[0024] Polystyrene aminomethylated (PAM) resin, BOC-protected aminoacids from Novabiochem, 2-(1H benzotriazole-yl)-1,3,3,4-tetramethyluronium hexafluorophosphate (HBTU) from Phase Separations Ltd, Trifluoroacetic acid (TFA) from Halocarbon Products Corporation, hydrogen fluoride gas (HF) from BOC, diisopropyl ethyl amine (DIEA) from Fluka and dimethylformamide (DMF) from Rathburn were all used as received. The protected lipidic aminoacids were synthesised and purified in our laboratory as described in Gibbons, WA, et al (1990) Liebigs Ann.Chem. 1177-1183.

#### Example 1

[0025] Solid phase peptide synthetic methods were used employing a polyacrylamide resin having a degree of substitution of 0.46 mmol/g resin. The reaction sequence is shown in flow diagram Figure 1 the step involving protection of Boc was performed in 100% trifluoroacetic acid. Couplings of pendant amine groups on the bound compound with carboxylic acid groups of amino acid reagents having protected amine groups was achieved using a three fold excess of HBTU activated Boc-amino acids in dimethylformamide in the presence of diisopropylethyl amine. Acidulation of

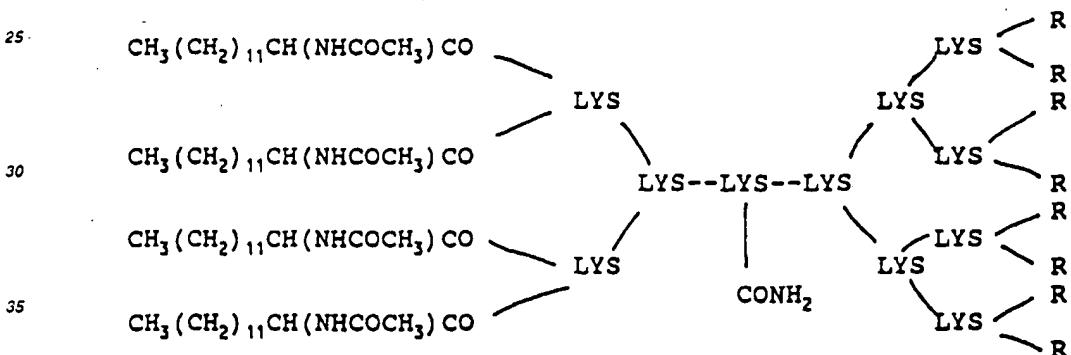
deprotected Boc group of lipoamino acid was carried out in the presence of diisopropylethyl amine. Deprotection of the Fmoc group to form the second dendron was carried out by a suitable system.

[0026] The resin peptide was carefully flow washed before and after each deprotection step. The final product was washed with dichloromethane and dried in air. The peptide was removed from the resin support with a high HF method 5 2 g resin peptide, 20 ml HF, 1.5 hour at -5°C) to yield the crude peptide which was dissolved in 95% acetic acid solution and lyophilised.

Purification

[0027] Analytical HPLC separation of the synthesised dendrimers was carried out on a 25 cm Vydac C<sub>18</sub> RAC column 10 with 5 μm pore size and 4.6 mm internal diameter. Following standard degassing techniques, particulate matter was removed from HPLC grade acetonitrile and water using membrane filters. Analytical separation was achieved with a solvent gradient beginning with 0% acetonitrile, increasing to 60% acetonitrile at 20 min. maintaining at this concentration for 20 min and decreasing steadily to 0% acetonitrile for 10 min at a constant flow of 1.2 ml min<sup>-1</sup>. For preparative 15 separation a TSK-GEL preparative C<sub>18</sub> column with 10 μm pore size and 2.5 cm internal diameter was used. Separation was achieved with a solvent gradient beginning with 0% acetonitrile, increasing constantly to 18% acetonitrile at 60 min then 60% acetonitrile at 80 min, staying at this concentration for further 30 min and decreasing steadily to 0% acetonitrile for 30 min at a constant flow of 8 ml min<sup>-1</sup>. The gradient was effected by two microprocessor-controlled Gilson 302 single piston pumps. Compounds were detected with a Waters 486 tunable absorbance detector at 214 20 nm or a Holochrome UV-VIS detector 220 nm. Mass spectra were run on VG Analytical Tofspec instrument, using matrix assisted laser desorption (MALD) ionisation at a wavelength of 337 nm generated by a nitrogen laser.

[0028] Compound synthesis using the general technique mentioned above had the following general structure:



[0029] The compounds synthesised have the values for the number of lipid residues and the number of primary 40 amine groups as well as the molecular weight shown in Table 1.

TABLE 1

Compound	R	Levels of dendritically linked lys residues in 2nd dendron	No 1° amine groups	MW
1.1	NH <sub>2</sub>	3	8	2500
1.2	Lys(NH <sub>2</sub> ) <sub>2</sub>	4	16	3526
1.3	Lys(Lys(NH <sub>2</sub> ) <sub>2</sub> ) <sub>2</sub>	5	32	5577

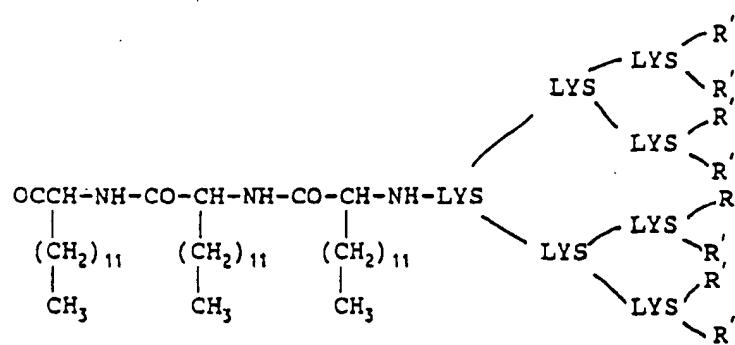
Comparative Example 1

[0030] Using the same general techniques described above in relation to Example 1, but omitting the Fmoc strategy, 45 compounds having the general formula shown below were produced. Thus the process involved three sequential steps to provide a linear tripeptide of lipoamino acid units bound to the glycine group attached to the resin, followed by a step of adding a Boc Lys (Boc) OH unit to the third lipoamino acid unit, followed by deprotection of both the amine groups of lysine and addition of sequential dendritically linked lysine moieties.

5

10

15



20

TABLE 2

25

Compound	R'	Levels of dendritically linked Lys (n)	No 1° amine groups	MW
1.4	NH <sub>2</sub>	3	8	1590
1.5	Lys(NH <sub>2</sub> ) <sub>2</sub>	4	16	2615
1.6	Lys(Lys(NH <sub>2</sub> ) <sub>2</sub> ) <sub>2</sub>	5	32	4666
1.7	CH <sub>2</sub> OH	3	0	3390

30

**Rat Erythrocyte Lysis Studies**  
 [0032] Fresh blood was obtained from rats through cardiac puncture, collected in heparinised tubes and centrifuged at 1,000 g for 15 minutes at 4°C. The supernatant, was discarded, the volume was made up to 10 ml with chilled phosphate buffered saline (PBS). The suspension was centrifuged again and the PBS washing step was repeated twice. Finally, the supernatant was removed and the cell pellet resuspended up to 2% w/v in chilled PBS. 100 µl of samples of compounds 1.1-1.7 of different dilutions were added in flat bottomed Elisa plate. 1% w/v of Triton X 100 was used as the control (100% lysis). 100 µl of erythrocyte suspension was added and incubated for 1h, 5h and 24 hrs. At different time intervals these plates were removed and the suspensions centrifuged. 100 µl of the supernatant was removed and placed into fresh Elisa plate and the absorbance was measured at 545 nm with PBS as blank. The % population lysis was calculated by using the formula

40

$$\text{Percentage population lysis} = (\text{Absorbance}/\text{control (triton) absorbance}) 100.$$

45

**Results**  
 [0033] The toxicity of compounds 1.1-1.7 were compared with linear polylysine of two different molecular weights (34,000 and 1000-4000). Triton X100 was used as positive control. The higher M.W. polylysine had a concentration independent toxicity 35.7% to 54.2% of percent population lysis was observed between the concentrations 1 µg/ml to 30 µg/ml. The lower M.W. polylysine was found to be almost nontoxic.

50

[0034] Red blood cellysis studies indicated that compounds 1.4-1.6 were non toxic at the low concentration of 1 µg/ml after 24 hrs where as at higher concentrations (above 20 µg/ml) these compounds were toxic even after one hour incubation. All compounds 1.1-1.6 had concentration dependent toxicity.

55

[0035] The toxicity studies of compounds 1.1 to 1.3 showed that the toxicity is dependent on the ratio of the lipophilic groups to the number of amino groups attached to the molecule. Compound 1.1, which contained 8 amino groups found to be less toxic than similar compounds having 16 and 32 amino groups (1.2 and 1.3) were less toxic than comparative 1.4 to 1.6. This indicates that although compounds 1.1 to 1.3 were bulkier, the position of attachment of lipo amino acid makes them less toxic.

[0036] Compound 1.7 which contained 3 lipo amino acid chain attached consecutively was non toxic at the concentration of 30  $\mu$ g/ml up to 5 hours incubation, indicating that the toxicity is not due to the presence of lipo amino acid.  
 [0037] The results are illustrated graphically in Figures 2 and 3.

5

## Claims

1. A dendritic compound comprising a core including a focal group from which at least two dendrons extend, each dendron comprising dendritically linked amino acid units I

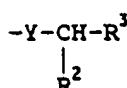
10



15

in which  $R^1$  is  $C_{1-6}$ -alkylene and X is selected from the group consisting of -O-, -S-, -NH- and -CO-, and each unit of a dendron may have the same groups  $R^1$  and X, and in which a first dendron has n (where n is 2) levels of dendritically linked amino acid units and  $2^n$  terminal branches, to p (where p is at least 2) of which terminal branches there are linked anchor groups

20



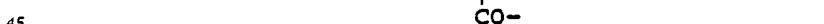
25

where Y is selected from -CO-, -NH-, -O- and -S-, provided that at least one of X and Y is -CO-,

$R^2$  is an organic group containing at least one  $C_{6-24}$ -alkyl, -alkenyl, or -alkynyl group,  
 R<sup>3</sup> is selected from the group consisting of hydrogen, amine, blocked amine, hydroxyl,  $C_{1-24}$  alkoxy, thiol,  
 30 COOH, or an organic group containing at least one  $C_{6-24}$ -alkyl, alkenyl or -alkynyl group,  $C_{1-6}$ -alkanoyloxy, or  
 $C_{1-6}$ -alkanamido,

and in which a second dendron has m (where m is in the range 3 to 5) levels of dendritically linked amino acid units of the formula I above in which the groups  $R^1$  and X may be the same as or different to one another and the same as or different to those of the amino acid units in the first dendron and  $2^m$  terminal branches, each of which is either unconjugated and is a group selected from  $NH_2$ ,  $N^+H_2R^{11}$ , in which  $R^{11}$  is hydrogen or  $C_{1-4}$  alkyl, COOH,  $COO^-$ , OH or SH, or is conjugated via the terminal -X-, -NH- or -CO- group to a group  $R^{12}$  where  $R^{12}$  is a methylol group, an active ligand or an organic group comprising a sugar moiety.

35 40 2. A compound according to claim 1 in which the focal group is an amino acid unit, having the formula I



45

in which X and  $R^1$  are as defined above.

50 3. A compound according to claim 2 in which X in the focal group is -CO- or -NH-.

4. A compound according to claim 3 in which X in the focal group is -NH-.

5. A compound according to claim 4 in which the focal group is formed from lysine or ornithine, that is  $R^1$  is  $(CH_2)_4$  or  $(CH_2)_3$ .

55 6. A compound according to any preceding claim in which  $2^n = p$ .

7. A compound according to any preceding claim in which each terminal branch of the second dendron is -NH<sub>2</sub> or

-N+H<sub>2</sub>R<sup>11</sup>.

8. A compound according to any preceding claim in which in each of the groups of the formula I, the groups R<sup>1</sup> and X are the same.

5

9. A compound according to claim 8 in which X is -NH- and R<sup>1</sup> is -(CH<sub>2</sub>)<sub>4</sub> or -(CH<sub>2</sub>)<sub>3</sub>-.

10

10. A compound according to any preceding claim which is bound to a resin support through the focal unit of the core.

11

11. A compound according to claim 10 which is bound to the support via a spacer.

12

12. A composition comprising a compound of any preceding claim.

13

13. A pharmaceutical composition comprising a pharmaceutical excipient and a compound according to any of claims 1 to 9.

14

14. A method of synthesis in which a focal reagent which has two reactive groups is reacted in a first series of first dendron producing steps as follows:

20

1. an amino acid reagent of the formula II

25



in which R<sup>1</sup> and X are as defined in claim 1

30

R<sup>14</sup> is H when X is -O-, -S- or -NH-,

OH when X is -CO-, or

is a protecting group,

R<sup>15</sup> is a carboxylic acid protecting group, hydroxyl, or a carboxylic acid activating group,

R<sup>16</sup> is H, an amine protecting group or an amine activating group,

35

provided that at least two of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than an activating group and at least one of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than a protecting group, is reacted

with the focal reagent, optionally after a step in which the desired reactive group of the focal reagent is deprotected and/or activated whereby the reactive group on the focal reagent reacts with one of the groups R<sup>14</sup>X-, R<sup>15</sup>-CO- and R<sup>16</sup>-NH-;

40

2. a second step in which both unreacted groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH- of the product of the preceding step are, if necessary, deprotected and/or activated and reacted with at least two equivalents of a trifunctional reagent having the general formula II,

45



50

in which the groups R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> are as defined in step 1 and are the same or different as in the trifunctional reagent used in step 1;

3. One repeat of step 2, using at least four equivalents of trifunctional reagent of the general formula II; and

4. An anchor group attachment step in which at least two of the four groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH- are, if necessary, deprotected and/or activated, and reacted with a lipophilic reagent of the formula III

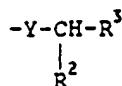
55



5

in which Y, R<sup>2</sup> and R<sup>3</sup> are as defined above, and R<sup>17</sup> is OH or a carboxylic acid activating group, where Y is -CO- or R<sup>17</sup> is H or an amine, hydroxyl or thiol activating group, respectively, where Y is -NH-, -O- or -S-, whereby the said at least two groups react with R<sup>17</sup>Y- to conjugate

10



15

groups to the X, CO- or NH-; and

a second dendron forming series of reaction steps in which

20

1. the other of the reactive groups of the focal reagent is, in a step separate to step 1 of the first series of first dendron producing steps,  
reacted with an amino acid reagent of the formula II

25



30

in which R<sup>1</sup> and X are as defined above,

R<sup>14</sup> is H when X is -O-, -S- or -NH-,  
OH when X is -CO-, or  
is a protecting group,

35

R<sup>15</sup> is a carboxylic acid protecting group, hydroxyl, or a carboxylic acid activating group,  
R<sup>16</sup> is H, an amine protecting group or an amine activating group,

40

provided that at least two of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than an activating group and at least one of R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> is other than a protecting group,

45

optionally after a step in which the desired reactive group of the focal reagent and/or one of the groups -XR<sup>14</sup>, -COR<sup>15</sup> and -NHR<sup>16</sup> is deprotected and/or activated whereby the other of the reactive groups on the focal reagent reacts with one of the groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH-;

50

2. a second step in which both unreacted groups R<sup>14</sup>X-, R<sup>15</sup>CO- and R<sup>16</sup>NH- of the product of the preceding step are, if necessary, deprotected and/or activated and reacted with at least two equivalents of a trifunctional reagent having the general formula II,

55

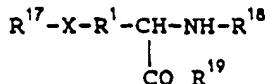


in which the groups R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> and R<sup>16</sup> are as defined in step 1 and are the same or different as in the trifunctional reagent used in step 1;

60

3. (m-1) repeats of step 2, using in each case at least 2<sup>(r+1)</sup> equivalents of trifunctional reagent for the r<sup>th</sup> repeat of step 2, until m levels of dendritically linked amino acids have been formed, where m is in the range 3 to 5.

15. A method according to claim 14 involving a preliminary step of reacting a focal reagent of the formula VI



VI

5

in which  $\text{R}^{17}$ ,  $\text{R}^{18}$  and  $\text{R}^{19}$  are selected from the same groups as  $\text{R}^{14}$ ,  $\text{R}^{16}$  and  $\text{R}^{15}$ , respectively, as defined in claim 14, with a substrate having a pendant group which is capable of reacting with one of the groups  $-\text{XR}^{17}$ ,  $-\text{NHR}^{18}$  and  $-\text{COR}^{19}$ , optionally after deprotection and/or activation of the said pendant group, whereby the focal reagent is bound to the substrate.

10

16. A method according to claim 15 in which the substrate is an immobile support, preferably a resin, more preferably a polyacrylamide-based resin.

15

17. A method according to claim 16 in which the resin has pendant amine groups and in which the group  $-\text{COR}^{19}$  is reacted with said pendant amine groups in the presence of an activating compound to form a peptide bond.

18. A method according to claim 17 in which  $\text{R}^{17}$  and  $\text{R}^{18}$  are each different amine protecting groups.

20

19. A method according to any of claims 14 to 18 in which in each of the steps in each respective series the reagent of the formula II is the same, preferably in which the reagent of the formula II is the same for each series.

20. A method according to claim 19 in which  $\text{X}$  is  $-\text{NH-}$  and in which the groups  $\text{R}^{14}$  and  $\text{R}^{15}$  are the same amino protecting groups.

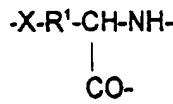
25

#### Patentansprüche

30

1. Dendritische Verbindung, umfassend einen Kern, der eine fokale Gruppe einschließt, von der mindestens zwei Dendrons ausgehen, wobei jedes Dendron dendritisch verknüpfte Aminosäureeinheiten I

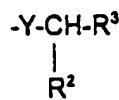
35



35

umfaßt, worin  $\text{R}^1$   $\text{C}_{1-6}$ -Alkylen ist und  $\text{X}$  aus der Gruppe, bestehend aus  $-\text{O-}$ ,  $-\text{S-}$ ,  $-\text{NH-}$  und  $-\text{CO-}$ , ausgewählt ist und jede Einheit eines Dendrons die gleichen Gruppen  $\text{R}^1$  und  $\text{X}$  aufweisen kann, und worin ein erstes Dendron  $n$  (wobei  $n$  2 ist) Ebenen von dendritisch verknüpften Aminosäureeinheiten und  $2^n$  endständige Zweige aufweist, wobei mit  $p$  (wobei  $p$  mindestens 2 ist) endständigen Zweigen Ankergruppen

45



45

verknüpft sind, worin  $\text{Y}$  aus  $-\text{CO-}$ ,  $-\text{NH-}$ ,  $-\text{O-}$  und  $-\text{S-}$  ausgewählt ist, mit der Maßgabe, daß mindestens eines von  $\text{X}$  und  $\text{Y-CO-}$  ist,

50

$\text{R}^2$  eine organische Gruppe ist, die mindestens eine  $\text{C}_{6-24}$ -Alkyl-, -Alkenyl- oder -Alkinylgruppe enthält,  $\text{R}^3$  aus der Gruppe, bestehend aus Wasserstoff, Amin, blockiertem Amin, Hydroxyl,  $\text{C}_{1-24}$ -Alkoxy, Thiol,  $\text{COOH}$  oder einer organischen Gruppe, die mindestens eine  $\text{C}_{6-24}$ -Alkyl-, -Alkenyl oder -Alkinylgruppe,  $\text{C}_{1-6}$ -Alkanoyloxy oder  $\text{C}_{1-6}$ -Alkanamido enthält, ausgewählt ist,

55

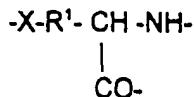
und worin ein zweites Dendron  $m$  (wobei  $m$  im Bereich von 3 bis 5 liegt) Ebenen von dendritisch verknüpften Aminosäureeinheiten der Formel I oben, worin die Gruppen  $\text{R}^1$  und  $\text{X}$  gleich oder verschieden voneinander und gleich denjenigen der Aminosäureeinheiten im ersten Dendron oder davon verschieden sein können, und  $2^m$  end-

ständige Zweige aufweist, von denen ein jeder entweder unkonjugiert ist und eine Gruppe darstellt, die aus  $\text{NH}_2$ ,  $\text{N}^+\text{H}_2\text{R}^{11}$ , worin  $\text{R}^{11}$  Wasserstoff oder C<sub>1-4</sub>-Alkyl, COOH,  $\text{COO}^-$ , OH oder SH ist, ausgewählt ist, oder über die endständige -X-, -NH- oder -CO-Gruppe mit einer Gruppe  $\text{R}^{12}$  konjugiert ist, wobei  $\text{R}^{12}$  eine Methylolgruppe, ein aktiver Ligand oder eine organische Gruppe ist, die eine Zuckergruppe umfaßt.

5

2. Verbindung nach Anspruch 1, worin die lokale Gruppe eine Aminosäureeinheit der Formel I

10



I

15

ist, worin X und  $\text{R}^1$  wie oben definiert sind.

15

3. Verbindung nach Anspruch 2, worin X in der lokalen Gruppe -CO- oder -NH- ist.

4. Verbindung nach Anspruch 3, worin X in der lokalen Gruppe -NH- ist.

20 5. Verbindung nach Anspruch 4, worin die lokale Gruppe von Lysin oder Ornithin gebildet wird, d.h.,  $\text{R}^1$  ist  $-(\text{CH}_2)_4-$  oder  $-(\text{CH}_2)_3-$ .

6. Verbindung nach irgendeinem der vorhergehenden Ansprüche, worin  $2^n = p$  ist.

25 7. Verbindung nach irgendeinem der vorhergehenden Ansprüche, worin jeder endständige Zweig des zweiten Dendrons -NH<sub>2</sub> oder -N<sup>+</sup>H<sub>2</sub>R<sup>11</sup> ist.

8. Verbindung nach irgendeinem der vorhergehenden Ansprüche, worin die Gruppen  $\text{R}^1$  und X in jeder der Gruppen der Formel I gleich sind.

30 9. Verbindung nach Anspruch 8, worin X -NH- ist und  $\text{R}^1$   $-(\text{CH}_2)_4-$  oder  $-(\text{CH}_2)_3-$  ist.

10. Verbindung nach irgendeinem der vorhergehenden Ansprüche, welche über die lokale Einheit des Kerns an einen Harzträger gebunden ist.

35 11. Verbindung nach Anspruch 10, welche über einen Spacer an den Träger gebunden ist.

12. Zusammensetzung, umfassend eine Verbindung nach irgendeinem der vorhergehenden Ansprüche.

40 13. Pharmazeutische Zusammensetzung, umfassend einen pharmazeutischen Exzipienten und eine Verbindung nach irgendeinem der Ansprüche 1 bis 9.

14. Syntheseverfahren, worin ein lokales Reagenz, welches zwei reaktive Gruppen aufweist, umgesetzt wird in einer ersten Folge von Schritten zur Bildung eines ersten Dendrons wie folgt:

45

1. ein Aminosäurerereagenz der Formel II

50



II

55

worin  $\text{R}^1$  und X wie in Anspruch 1 definiert sind,

$\text{R}^{14}$  H ist, wenn X -O-, -S- oder -NH- ist,

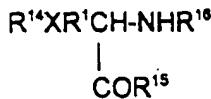
OH ist, wenn X -CO- ist, oder eine Schutzgruppe ist,

$\text{R}^{15}$  eine Carbonsäure-Schutzgruppe, Hydroxyl oder eine Carbonsäure-aktivierende Gruppe ist,

$R^{16}H$ , eine Amin-Schutzgruppe oder eine Amin-aktivierende Gruppe ist, vorausgesetzt, daß mindestens zwei von  $R^{14}$ ,  $R^{15}$  und  $R^{16}$  keine aktivierende Gruppe sind und mindestens eines von  $R^{14}$ ,  $R^{15}$  und  $R^{16}$  keine Schutzgruppe ist,

5 wird mit dem lokalen Reagenz umgesetzt, gegebenenfalls nach einem Schritt, in dem die gewünschte reaktive Gruppe des lokalen Reagenz von der Schutzgruppe befreit und/oder aktiviert wird, wodurch die reaktive Gruppe auf dem lokalen Reagenz mit einer der Gruppen  $R^{14}X$ -,  $R^{15}CO$ - und  $R^{16}NH$ - reagiert;

10 2. ein zweiter Schritt, in dem beide nicht umgesetzten Gruppen  $R^{14}X$ -,  $R^{15}CO$ - und  $R^{16}NH$ - des Produkts des vorhergehenden Schritts erforderlichenfalls von der Schutzgruppe befreit und/oder aktiviert werden und mit mindestens zwei Äquivalenten eines trifunktionellen Reagenz der allgemeinen Formel II



15 umgesetzt werden, worin die Gruppen  $R^1$ ,  $R^{14}$ ,  $R^{15}$  und  $R^{16}$  wie in Schritt 1 definiert sind und die gleichen wie in dem in Schritt 1 eingesetzten trifunktionellen Reagenz oder davon verschieden sind;

20 3. eine Wiederholung von Schritt 2, wobei mindestens vier Äquivalente des trifunktionellen Reagenz der allgemeinen Formel II eingesetzt werden; und

25 4. ein Ankergruppen-Verknüpfungsschritt, worin mindestens zwei der vier Gruppen  $R^{14}X$ -,  $R^{15}CO$ - und  $R^{16}NH$ - erforderlichenfalls von der Schutzgruppe befreit und/oder aktiviert werden und mit einem lipophilen Reagenz der Formel III

30 umgesetzt werden,  
worin  $Y$ ,  $R^2$  und  $R^3$  wie oben definiert sind und  $R^{17}OH$  oder eine Carbonsäure-aktivierende Gruppe ist, wenn  $Y-CO$ - ist,  
35 oder  $R^{17}H$  oder eine Amin-, Hydroxyl- oder Thiol-aktivierende Gruppe ist, wenn  $Y-NH$ -,  $-O$ - oder  $-S$ - ist,  
wodurch die genannten mindestens zwei Gruppen mit  $R^{17}Y$ - reagieren, um

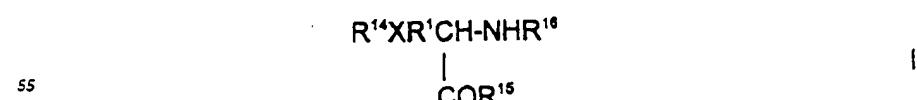
$-Y-CH-R^3$ -Gruppen



40 mit dem  $X$ ,  $CO$ - oder  $NH$ - zu konjugieren;

45 und einer Folge von Reaktionsschritten zur Bildung eines zweiten Dendrons, beinhaltend

50 1. daß die andere der reaktiven Gruppen des lokalen Reagenz in einem Schritt, welcher von Schritt 1 der ersten Folge von Schritten zur Bildung des ersten Dendrons getrennt ist, mit einem Aminosäurereagenz der Formel II



55 umgesetzt wird, worin  $R^1$  und  $X$  wie oben definiert sind,

R<sup>14</sup> H ist, wenn X -O-, -S- oder -NH- ist,  
 OH ist, wenn X -CO- ist, oder eine Schutzgruppe ist,  
 R<sup>15</sup> eine Carbonsäure-Schutzgruppe, Hydroxyl oder eine Carbonsäure-aktivierende Gruppe ist,  
 R<sup>16</sup> H, eine Amin-Schutzgruppe oder eine Amin-aktivierende Gruppe ist,  
 5 vorausgesetzt, daß mindestens zwei von R<sup>14</sup>, R<sup>15</sup> und R<sup>16</sup> keine Aktivierungsgruppe sind und mindestens  
 eines von R<sup>14</sup>, R<sup>15</sup> und R<sup>16</sup> keine Schutzgruppe ist;  
 gegebenenfalls nach einem Schritt, in dem die gewünschte reaktive Gruppe des fokalen Reagenz und/  
 oder eine der Gruppen -XR<sup>14</sup>, -COR<sup>15</sup>  
 10 und -NHR<sup>16</sup> von der Schutzgruppe befreit und/oder aktiviert wird, wodurch die andere der reaktiven Grup-  
 pen auf dem fokalen Reagenz mit einer der Gruppen R<sup>14</sup> X-, R<sup>15</sup>CO- und R<sup>16</sup>NH- reagiert;

2. einen zweiten Schritt, in dem beide nicht umgesetzten Gruppen R<sup>14</sup>X-, R<sup>15</sup>CO- und R<sup>16</sup>NH- des Produkts  
 15 des vorhergehenden Schritts erforderlichenfalls von der Schutzgruppe befreit und/oder aktiviert werden und  
 mit mindestens zwei Äquivalenten eines trifunktionellen Reagenz der allgemeinen Formel II

15

16

17

18

19

20



21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

149

150

151

152

153

154

155

156

157

158

159

160

161

162

163

164

165

166

167

168

169

170

171

172

173

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

228

229

2210

2211

2212

2213

2214

2215

2216

2217

2218

2219

2220

2221

2222

2223

2224

2225

2226

2227

2228

2229

22210

22211

22212

22213

22214

22215

22216

22217

22218

22219

22220

22221

22222

22223

22224

22225

22226

22227

22228

22229

222210

222211

222212

222213

222214

222215

222216

222217

222218

222219

222220

222221

222222

222223

222224

222225

222226

222227

222228

222229

2222210

2222211

2222212

2222213

2222214

2222215

2222216

2222217

2222218

2222219

2222220

2222221

2222222

2222223

2222224

2222225

2222226

2222227

2222228

2222229

22222210

22222211

22222212

22222213

22222214

22222215

22222216

22222217

22222218

22222219

22222220

22222221

22222222

22222223

22222224

22222225

22222226

22222227

## Revendications

1. Composé dendritique comprenant un noyau incluant un groupe focal d'où s'étendent au moins deux dendrons, chaque dendron comprenant des unités d'aminoacide I liées de manière dendritique

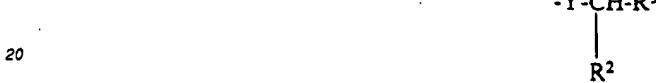
5



10

où  $R^1$  est alkylène en  $C_{1-6}$  et  $X$  est choisi dans le groupe consistant en  $-O-$ ,  $-S-$ ,  $-NH-$  et  $-CO-$ , et chaque unité d'un dendron peut avoir les mêmes groupes  $R^1$  et  $X$ , et où un premier dendron a  $n$  (où  $n$  est 2) niveaux d'unités d'aminoacide liées de manière dendritique et  $2^n$  branches terminales, branches terminales à  $p$  desquelles (où  $p$  est au moins 2) sont liés des groupes d'ancre

15



20

où  $Y$  est choisi parmi  $-CO-$ ,  $-NH-$ ,  $-O-$  et  $-S-$ , à condition qu'au moins l'un des groupes  $X$  et  $Y$  soit  $-CO-$ ,

25

$R^2$  est un groupe organique contenant au moins un groupe alkyle, alcényle ou alcynyle en  $C_{6-24}$ ,  $R^3$  est choisi dans le groupe consistant en l'hydrogène, amine, amine bloquée, hydroxyle, alcoxy en  $C_{1-24}$ , thiol,  $COOH$  ou un groupe organique contenant au moins un groupe alkyle, alcényle ou alcynyle en  $C_{6-24}$ , alcanoxy en  $C_{1-6}$  ou alcanamido en  $C_{1-6}$ .

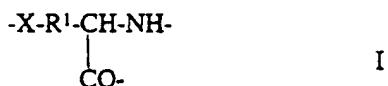
30

et où un second dendron a  $m$  (où  $m$  est dans le domaine de 3 à 5) niveaux d'unités d'aminoacide liées de manière dendritique de formule I ci-dessus où les groupes  $R^1$  et  $X$  peuvent être identiques ou différents l'un de l'autre et identiques ou différents de ceux des unités d'aminoacide dans le premier dendron et  $2^m$  branches terminales, dont chacune est non conjuguée et est un groupe choisi parmi  $NH_2$ ,  $N^+H_2R^{11}$  où  $R^{11}$  est l'hydrogène ou alkyle en  $C_{1-4}$ ,  $COOH$ ,  $COO^-$ ,  $OH$  ou  $SH$ , ou est conjuguée par le groupe  $-X$ ,  $-NH-$  ou  $-CO-$  terminal à un groupe  $R^{12}$  où  $R^{12}$  est un groupe méthylol, un ligand actif ou un groupe organique comprenant une entité glucidique.

35

2. Composé selon la revendication 1, où le groupe focal est une unité d'aminoacide ayant la formule I

40



45

où  $X$  et  $R^1$  sont définis comme ci-dessus.

45

3. Composé selon la revendication 2, où  $X$  dans le groupe focal est  $-CO-$  ou  $-NH-$ .

50

4. Composé selon la revendication 3, où  $X$  dans le groupe focal est  $-NH-$ .

55

5. Composé selon la revendication 4, où le groupe focal est constitué par de la lysine ou de l'ornithine, c'est-à-dire que  $R^1$  est  $(CH_2)_4$  ou  $(CH_2)_3$ .

60

6. Composé selon l'une quelconque des revendications précédentes, où  $2^n = p$ .

65

7. Composé selon l'une quelconque des revendications précédentes, où chaque branche terminale du second dendron est  $-NH_2$  ou  $-N^+H_2R^{11}$ .

70

8. Composé selon l'une quelconque des revendications précédentes, où, dans chacun des groupes de formule I, les

groupes R<sup>1</sup> et X sont les mêmes.

9. Composé selon la revendication 8, où X est -NH- et R<sup>1</sup> est -(CH<sub>2</sub>)<sub>4</sub> ou -(CH<sub>2</sub>)<sub>3</sub>-.

5 10. Composé selon l'une quelconque des revendications précédentes, qui est lié à un support résinique par l'unité focale du noyau.

11. Composé selon la revendication 10, qui est lié au support par le biais d'un espaceur.

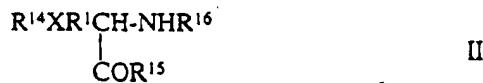
10 12. Composition comprenant un composé selon l'une quelconque des revendications précédentes.

13. Composition pharmaceutique comprenant un excipient pharmaceutique et un composé selon l'une quelconque des revendications 1 à 9.

15 14. Procédé de synthèse où un réactif focal qui a deux groupes réactifs est mis à réagir dans une première série d'étapes de production d'un premier dendron de la manière suivante :

1. un réactif aminoacide de formule II

20



25 où R<sup>1</sup> et X sont définis comme dans la revendication 1

R<sup>14</sup> est H quand X est -O-, -S- ou -NH-,

OH quand X est -CO-, ou

est un groupe protecteur,

30 R<sup>15</sup> est un groupe protecteur d'acide carboxylique, un groupe hydroxyle ou un groupe activateur d'acide carboxylique,

R<sup>16</sup> est H, un groupe protecteur d'amine ou un groupe activateur d'amine,

35 à condition qu'au moins deux des groupes R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> ne soient pas un groupe activateur et qu'au moins l'un des groupes R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> ne soit pas un groupe protecteur,

est mis à réagir avec le réactif focal, éventuellement après une étape au cours de laquelle le groupe réactif souhaité du réactif focal est déprotégé et/ou activé de sorte que le groupe réactif sur le réactif focal réagit avec l'un des groupes R<sup>14</sup>X-, R<sup>15</sup>-CO- et R<sup>16</sup>-NH- ;

40 2. une seconde étape au cours de laquelle les deux groupes R<sup>14</sup>X-, R<sup>15</sup>-CO- et R<sup>16</sup>-NH- qui n'ont pas réagi du produit de l'étape précédente sont, si nécessaire, déprotégés et/ou activés et mis à réagir avec au moins deux équivalents d'un réactif trifonctionnel ayant la formule générale II,

45

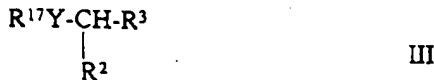


où les groupes R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> sont définis comme dans l'étape 1 et sont identiques ou différents de ceux du réactif trifonctionnel utilisé dans l'étape 1 ;

50 3. une répétition de l'étape 2 au moyen d'au moins 4 équivalents de réactif trifonctionnel de formule générale II ; et

4. une étape de fixation de groupes d'ancrage au cours de laquelle au moins deux des quatre groupes R<sup>14</sup>X-, R<sup>15</sup>-CO- et R<sup>16</sup>-NH- sont, si nécessaire, déprotégés et/ou activés, et mis à réagir avec un réactif lipophile de formule III

55

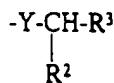


5

où Y, R<sup>2</sup> et R<sup>3</sup> sont définis comme ci-dessus, et R<sup>17</sup> est OH ou un groupe activateur d'acide carboxylique quand Y est -CO-, ou bien R<sup>17</sup> est H ou un groupe activateur d'amine, d'hydroxyle ou de thiol, respectivement, quand Y est -NH-, -O- ou -S-, de sorte que lesdits groupes au nombre d'au moins deux réagissent avec R<sup>17</sup>Y-pour conjuguer des groupes

10

15



à X, CO- ou NH- ; et

20

une seconde série d'étapes réactionnelles de formation d'un second dendron où

25

1. dans une étape séparée de l'étape 1 de la première série d'étapes de production d'un premier dendron, l'autre des groupes réactifs du réactif focal est mis à réagir avec un réactif aminoacide de formule II

30

35



où R<sup>1</sup> et X sont définis comme ci-dessus,

R<sup>14</sup> est H quand X est -O-, -S- ou -NH-,

OH quand X est -CO-, ou

est un groupe protecteur,

R<sup>15</sup> est un groupe protecteur d'acide carboxylique, un groupe hydroxyle ou un groupe activateur d'acide carboxylique,

R<sup>16</sup> est H, un groupe protecteur d'amine ou un groupe activateur d'amine,

à condition qu'au moins deux des groupes R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> ne soient pas un groupe activateur et qu'au moins l'un des groupes R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> ne soit pas un groupe protecteur,

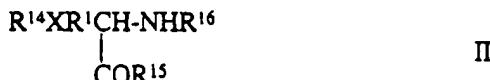
40

éventuellement après une étape au cours de laquelle le groupe réactif souhaité du réactif focal et/ou l'un des groupes -XR<sup>14</sup>, -COR<sup>15</sup> et -NHR<sup>16</sup> est déprotégé et/ou activé de sorte que l'autre des groupes réactifs du réactif focal réagit avec l'un des groupes R<sup>14</sup>X-, R<sup>15</sup>-CO- et R<sup>16</sup>-NH- ;

45

2. une seconde étape au cours de laquelle les deux groupes R<sup>14</sup>X-, R<sup>15</sup>-CO- et R<sup>16</sup>-NH- qui n'ont pas réagi du produit de l'étape précédente sont, si nécessaire, déprotégés et/ou activés et mis à réagir avec au moins deux équivalents d'un réactif trifonctionnel ayant la formule générale II,

50



où les groupes R<sup>1</sup>, R<sup>14</sup>, R<sup>15</sup> et R<sup>16</sup> sont définis comme dans l'étape 1 et sont identiques ou différents de ceux du réactif trifonctionnel utilisé dans l'étape 1 ;

55

3. (m-1) répétitions de l'étape 2, utilisant dans chaque cas au moins 2<sup>(r+1)</sup> équivalents de réactif trifonctionnel pour la r<sup>ème</sup> répétition de l'étape 2, jusqu'à ce que m niveaux d'aminoacides liés de manière dendritique aient été formés, où m est dans le domaine de 3 à 5.

15. Procédé selon la revendication 14, mettant en jeu une étape préliminaire de réaction d'un réactif focal de formule VI

5



10 où  $\text{R}^{17}$ ,  $\text{R}^{18}$  et  $\text{R}^{19}$  sont choisis dans les mêmes groupes que  $\text{R}^{14}$ ,  $\text{R}^{16}$  et  $\text{R}^{15}$ , respectivement, tels que définis dans la revendication 14, avec un substrat ayant un groupe latéral qui est capable de réagir avec l'un des groupes - $\text{XR}^{17}$ , - $\text{NHR}^{18}$  et - $\text{COR}^{19}$ , éventuellement après déprotection et/ou activation dudit groupe latéral, de sorte que le réactif focal est lié au substrat.

15 16. Procédé selon la revendication 15, où le substrat est un support immobile, de préférence une résine, de préférence encore une résine à base de polyacrylamide.

17. Procédé selon la revendication 16, où la résine a des groupes amine latéraux et où le groupe - $\text{COR}^{19}$  est mis à réagir avec lesdits groupes amine latéraux en présence d'un composé activateur pour former une liaison peptidique.

20 18. Procédé selon la revendication 17, où  $\text{R}^{17}$  et  $\text{R}^{18}$  sont chacun des groupes protecteurs d'amine différents.

19. Procédé selon l'une quelconque des revendications 14 à 18 où, dans chacune des étapes dans chaque série respective, le réactif de formule II est le même, de préférence où le réactif de formule II est le même pour chaque série.

25 20. Procédé selon la revendication 19, où X est -NH- et où les groupes  $\text{R}^{14}$  et  $\text{R}^{15}$  sont les mêmes groupes protecteurs d'amino.

30

35

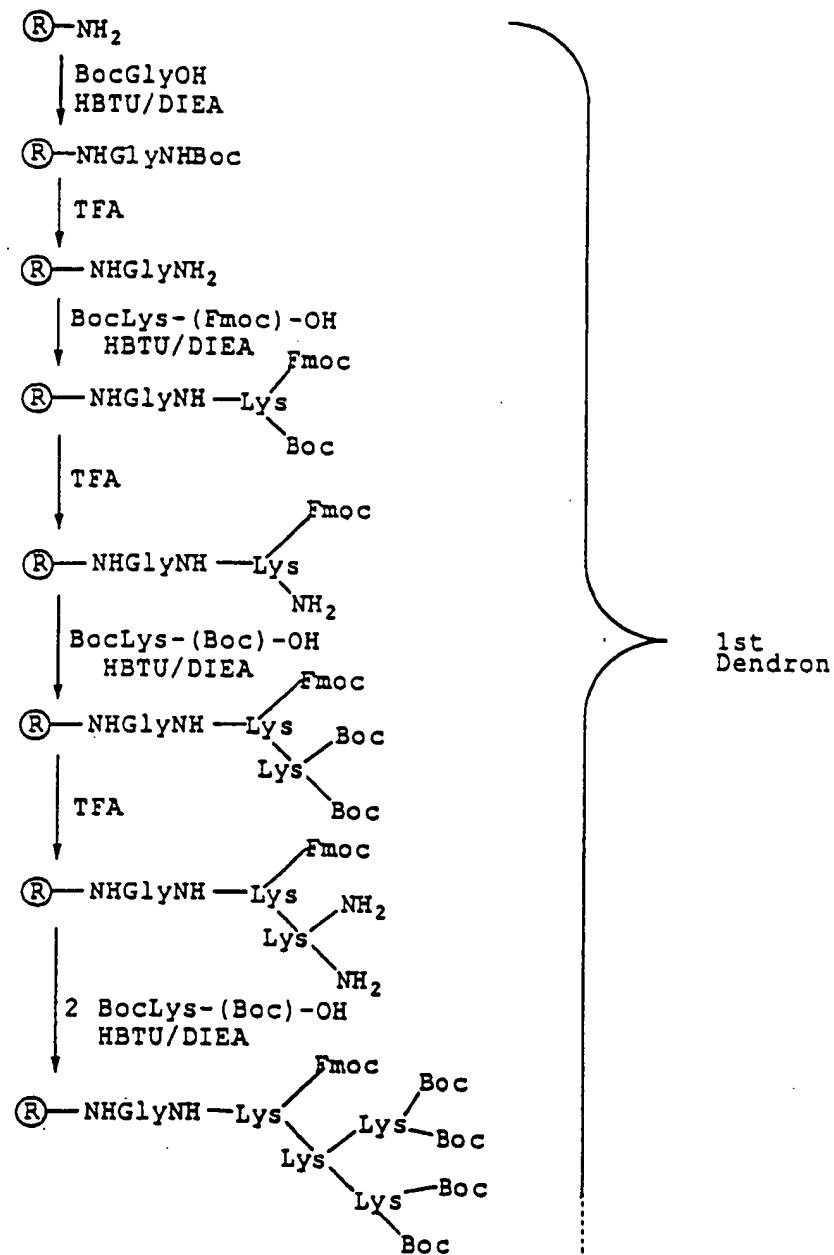


Figure 1.

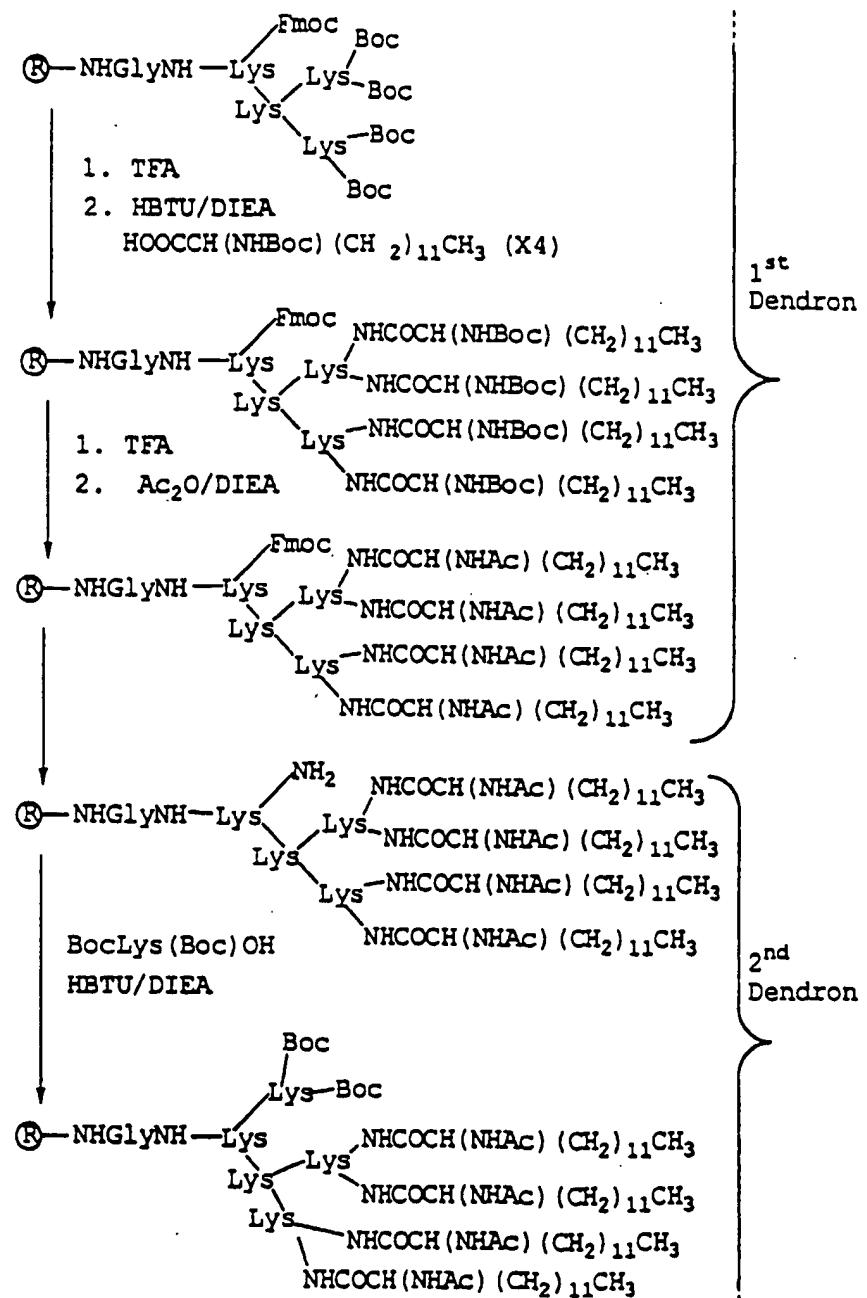


Figure 1 (cont.)

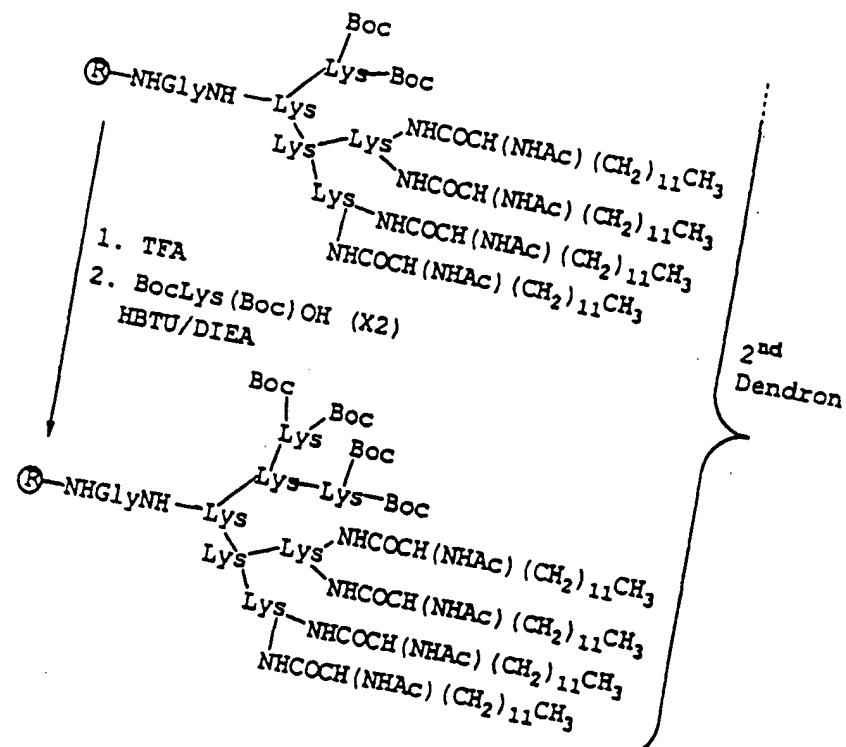


Figure 1 (cont.)

**Haemoglobin release after 1h. Incubation of rat erythrocytes with dendrimers**

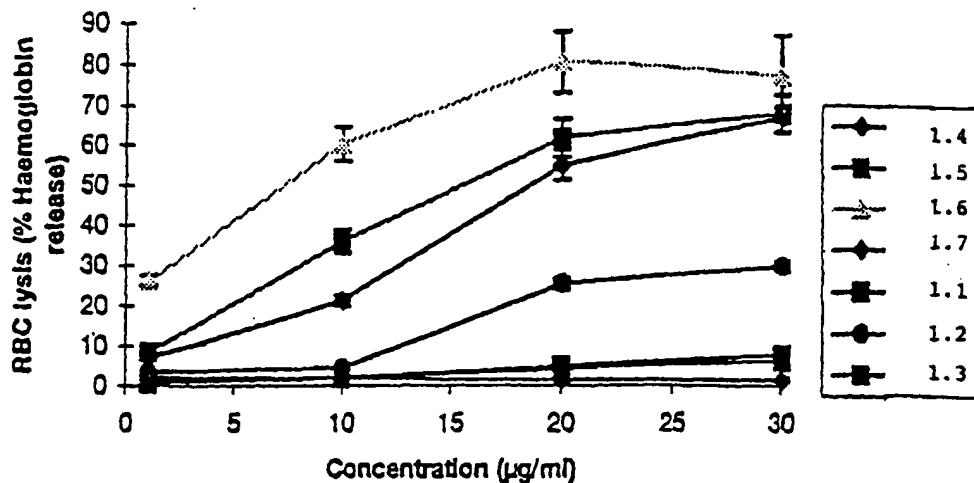


FIG 2

**Relationship between the number of amino groups and haemoglobin release at 20  $\mu\text{g/ml}$  after 1h. incubation**

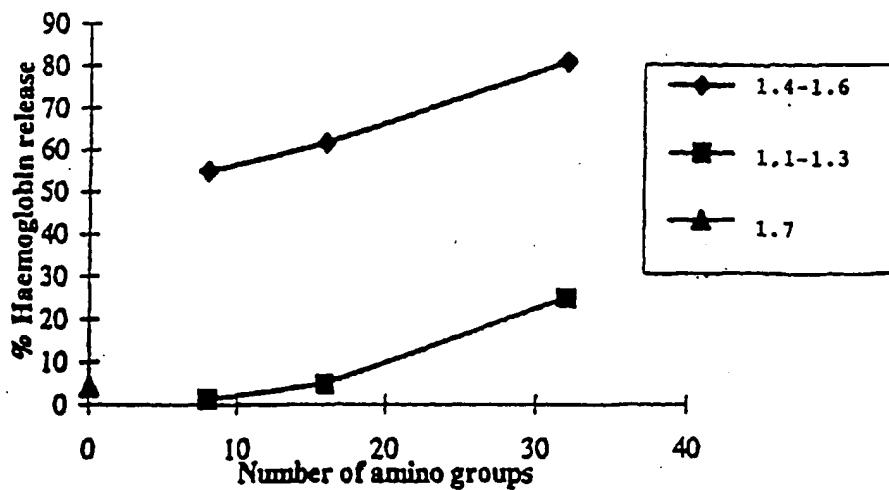


FIG 3